

GENETIC DIVERGENCE ASSESSMENT IN UPLAND COTTON (*Gossypium hirsutum* L.) USING VARIOUS STATISTICAL TOOLS

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Sustainable cotton production depends upon evolution of the crop genotypes with higher yield, improved fiber quality and better tolerance against biotic and abiotic stresses. A field experiment was conducted at department of plant breeding and genetics, University of Agriculture, Faisalabad to access genetic divergence among 23 different genotypes of upland cotton for various yield and fiber related traits. The data were analyzed by using simple correlation coefficient, metroglyph, principal component and cluster analysis. Results from present study showed strong positive correlation (0.97) of number of bolls with seed cotton yield and also positive correlation with lint index (0.35) and fiber length (0.30); whilst fiber strength showed negative correlation (-0.45) with seed cotton yield. Amongst the seven clusters named I, II, III, IV, V, VI and VII, formed through metroglyph analysis, cluster III had maximum individual genotypic scores for various yield and fiber related traits. Principal component analysis partitioned total variability of genotypes into four PCs with the PC-1 contributing the highest (35%) towards the total variability (82.2%) with positive factor loadings of yield related traits such as number of bolls, seed cotton yield and fiber length. *k*-means cluster analysis grouped various studied genotypes/lines into three distinct clusters. Cluster-II and cluster-III showed better values for seed cotton yield, number of bolls, seed index and fiber length. The information generated through using various statistical tools to assess genetic diversity may be useful in developing a better cotton breeding program.

Keywords: Genetic diversity, Metroglyph analysis, Principal Component Analysis, Cotton genotypes

INTRODUCTION

Cotton is most widely cultivated leading fiber crop in the world. Upland cotton (*Gossypium hirsutum* L.) meets 90% of worlds' cotton demands (Wendel *et al.*, 1992). Sustainable cotton production will depend upon the evolution of cotton varieties with superior fiber quality, higher yield and better tolerance against various environmental calamities. As changing climate has drastic effects on overall crop production, especially temperature extremes at germinating and maturity stages of a crop (Afzal *et al.*, 2015; Ahmad, 1999; Baloch *et al.*, 1999). Besides agronomic approaches to overcome these biotic and abiotic stresses (Bakhtavar *et al.*, 2015), it is vital to devise the genotypes with genetically superior quantitative and qualitative traits. Pakistani cotton producers however are facing rising production costs and static returns due to low productivity and prevalence of insect, pests and diseases (Haider *et al.*, 2007). Cotton is often cross pollinated crop which has ample amount of variation for important yield and fiber quality traits, however cotton breeders only used fraction of available variation from germplasm resources for modern cultivar development. Present day cotton cultivars

were developed through repeating cycles of reselection which has resulted in reduction of genetic variability (Bowman *et al.*, 2001). Taking in account the choice of good parents having broader genetic base for various yield and fiber quality traits is crucial for future cotton breeding programs (Esbroeck and Bowman, 1998). Various efforts had been made to uplift the overall production and profitability of cotton crop in the country. Knowledge of genetic divergence and association of various quantitative traits in cotton is vital to select genetically superior plants which may be utilized in breeding programs. As superior yield contributing traits (quantitative and/or qualitative) are the ultimate determinants of better growth and development of a crop plant, leading to higher grain yields (Noor *et al.*, 2016). The Anderson's Metroglyph is a simple technique which is useful for preliminary grouping of different genotypes in distinct groups on the basis of morphological characters. This technique has been used by many researchers viz. Shakeel *et al.* (2011) on cotton, Bhargava *et al.* (2009) on *Chenopodium* spp., Khan *et al.* (2005) on Brassica spp. and Rashid *et al.* (2007) on basmati rice lines to determine genetic diversity. Meanwhile, when partitioning of total variation is required, Principal

Component Analysis is most suitable statistical method that provides facilitation for obtaining appropriate parental lines in order to initiate successful breeding program (Akter *et al.*, 2009; Nazir *et al.*, 2013). Simple correlation coefficient analysis was also employed to obtain suitable associations among studied traits (Ali *et al.*, 2009). Therefore, present study was undertaken to analyze cotton genotypes by applying different statistical analysis with hypothesis to enable us classifying available germplasm in different clusters on the basis of their genetic diversity. The information obtained will be helpful in obtaining genotypes having potential in various quality and yield contributing traits to utilize them in further breeding programs.

MATERIALS AND METHODS

Plant material and site characteristics: Twenty-one cotton genotypes and two standard varieties having wider adaptability were analyzed under field conditions (2013-14). Delinted seeds of germplasm accessions were grown in a triplicate randomized complete block design (RCBD) at the experimental area of the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan. The recommended row to row 75 cm and plant to plant distance 30 cm, respectively were maintained. The germplasm utilized for the present set of experiment were homogeneous. All the recommended agronomic practices were carried out throughout the crop growing period.

Measurement of studied traits: For measuring the traits, five representative undamaged healthy plants were selected randomly from each germplasm accession and subsequently marked for identification. Genotypes were evaluated for

bolts per plant (NB), seed cotton yield (SCY), seed index (SI), lint index (LI) and boll weight (BW). Fiber quality parameters including fiber fineness (FF), fiber length (FL), fiber strength (FS) and fiber uniformity (FU) were measured by Spin lab HVI-900.

Analysis of data: The average data were subjected to basic statistics (descriptive), correlation analysis, cluster analysis, metroglyph analysis (Anderson, 1957) and principal component analysis (PCA) through statistical software SPSS version 19 and STATISTICA version 5.0 (Sneath and Sokal, 1973). Cluster analysis was employed by using K-means clustering.

RESULTS

Correlation: The basic statistics of studied traits demonstrated ample amount of variability was present among 23 cotton line/genotypes (Table 1). The results of simple correlation coefficient revealed positive association among studied traits (Table 2). Lint index showed positive association with seed index and it also exhibited a positive correlation with seed cotton yield, whilst for other traits it showed non-significant relation. Boll weight exhibited negative correlation with bolts per plant and also showed a negative association with fiber length. Whereas, bolts per plants were found to be positively associated with seed cotton yield and with fiber length, whilst it exhibited negative association with fiber strength. Fiber length and seed cotton yield were positively correlated; on the other hand fiber strength exhibited negative correlation with seed cotton yield.

Table 1: Descriptive statistics for various traits among 23 cotton genotypes.

Variable	Mean	SE Mean	SD	Variance	CV	Min.	Max.	Range
Lint index (g)	4.094	0.137	0.657	0.431	16.0	2.686	5.392	2.705
Seed index (g)	7.192	0.147	0.705	0.497	9.80	5.985	8.095	2.110
Boll weight (g)	3.066	0.070	0.337	0.1137	10.9	2.427	4.0985	1.671
Number of bolls	26.74	1.59	7.63	58.19	28.5	15.40	47.60	32.20
Fiber length (mm)	27.69	0.203	0.973	0.946	3.51	25.950	29.250	3.300
Fiber strength (g/tex)	28.37	0.427	2.049	4.197	7.22	21.650	30.750	9.100
Fiber Uniformity (%)	48.143	0.488	2.340	5.477	4.86	44.650	52.550	7.900
Fiber fineness (µg/inch)	4.8696	0.0443	0.2125	0.0452	4.36	4.4500	5.2500	0.800
Seed cotton yield (g)	81.80	4.04	19.37	375.01	23.67	56.61	146.63	90.02

Table 2: Simple correlation coefficients of various yield and fiber quality traits in upland cotton genotypes.















Traits	LI	SI	BW	NB	FL	FS	FU	FF
SI	0.821**							
BW	-0.070	0.235						
NB	0.347	0.051	-0.537*					
FL	0.298	0.120	-0.492*	0.420				
FS	-0.114	0.179	0.190	-0.428	-0.086			
FU	0.040	-0.001	-0.003	0.120	0.291	-0.142		
FF	0.247	0.142	-0.032	0.071	0.288	0.080	0.374	





SCY	0.415*	0.094	-0.380	0.967**	0.410*	-0.455*	0.156	0.126
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Table 3: Mean data and the scores (in brackets) of various quantitative traits of 23 upland cotton genotypes.

	LI	SI	BW	SCY	NB	FL	FS	FU	FF	Total
V1	4.35(3)	6.67(1)	3.53(3)	63.85(1)	18.50(1)	28.00(2)	30.80(3)	47.20(2)	4.80(2)	18
V2	4.78(3)	7.94(3)	3.41(3)	65.18(1)	19.30(1)	27.00(1)	29.10(2)	47.30(2)	4.80(2)	18
V3	4.62(3)	8.09(3)	3.18(2)	66.31(1)	20.50(1)	29.00(3)	30.40(3)	50.10(3)	5.10(3)	22
V4	4.15(2)	7.06(2)	3.04(2)	71.45(2)	22.70(1)	27.90(2)	28.10(2)	47.90(2)	5.20(3)	18
V5	4.25(2)	6.84(1)	3.21(3)	74.28(2)	25.50(2)	26.90(1)	26.20(1)	48.20(2)	5.00(2)	16
V6	4.54(3)	8.04(3)	3.15(2)	79.09(2)	23.50(2)	27.40(2)	30.70(3)	46.40(1)	5.20(3)	21
V7	3.99(2)	7.74(2)	3.14(2)	70.34(1)	23.40(2)	28.50(3)	27.40(1)	47.10(1)	4.90(2)	16
V8	3.63(1)	6.64(1)	2.86(1)	68.31(1)	21.70(1)	28.40(3)	29.60(3)	44.70(1)	4.60(1)	13
V9	3.88(2)	6.94(2)	2.79(1)	92.61(3)	36.70(3)	28.10(2)	29.60(3)	47.30(2)	4.90(2)	20
V10	3.89(2)	7.04(2)	3.00(2)	84.24(2)	28.50(2)	27.30(1)	30.70(3)	49.90(2)	5.00(3)	19
V11	3.75(1)	6.84(1)	2.90(1)	91.35(3)	30.80(3)	29.30(3)	28.90(2)	51.50(3)	5.30(3)	20
V12	3.83(2)	6.24(1)	2.98(1)	91.91(3)	31.30(3)	27.90(2)	21.70(1)	50.00(3)	4.70(1)	17
V13	3.80(1)	7.14(2)	3.13(2)	85.66(2)	28.40(2)	26.00(1)	27.90(1)	44.90(1)	5.00(2)	14
V14	3.70(1)	6.67(1)	3.21(3)	89.46(3)	28.40(2)	28.70(3)	27.10(1)	45.50(1)	5.10(3)	18
V15	3.74(1)	5.99(1)	3.62(3)	87.70(2)	27.90(2)	26.00(1)	27.90(1)	45.80(1)	4.70(1)	13
V16	4.00(2)	7.84(3)	3.37(3)	56.62(1)	15.40(1)	26.00(1)	29.10(2)	47.90(2)	4.70(1)	16
V17	3.40(1)	7.77(2)	3.70(3)	62.25(1)	18.00(1)	26.70(1)	29.20(3)	51.20(3)	4.80(2)	17
V18	3.87(2)	6.19(1)	3.23(3)	61.96(1)	17.20(1)	27.30(1)	28.60(2)	51.20(3)	4.90(2)	16
V19	5.24(3)	7.65(2)	2.99(2)	107.89(3)	37.30(3)	28.60(3)	27.40(1)	52.60(3)	5.10(3)	23
V20	5.04(3)	8.02(3)	2.98(1)	146.64(3)	47.60(3)	29.10(3)	25.30(1)	50.20(3)	5.00(2)	22
V21	4.62(3)	7.84(3)	2.90(1)	95.29(3)	33.80(3)	27.50(2)	28.30(2)	45.70(1)	4.70(1)	19
V22	3.74(1)	8.10(3)	2.90(1)	86.94(2)	30.10(3)	28.00(2)	29.00(2)	45.60(1)	4.50(1)	16
V23	5.23(3)	8.29(3)	3.13(2)	100.84(3)	32.20(3)	28.40(3)	27.90(1)	50.80(3)	4.60(3)	24

Table 4: Class interval for 9 quantitative traits in 23 genotypes of *Gossypium hirsutum* L.

Character	Low (1)	Medium (2)	Sign	High (3)	Sign
Number of bolls per plant	15.4–22.7	22.8–28.5		28.6–36.7	
Boll weight	2.86–2.98	2.98–3.17		3.17–3.70	
Seed cotton yield	56.6–70.3	70.3–87.70		87.7–146.6	
Lint index	2.92–3.80	3.81–4.25		4.26–5.24	
Seed index	5.99–6.84	6.85–7.77		7.78–8.29	
Fiber length	26–27.3	27.4–28.1		28.2–29.30	
Fiber strength	21.7–27.9	28.0–29.1		29.2–30.8	

Fiber fineness	5.1–5.2	4.8–5.0		4.5–4.7	
Fiber uniformity	44.3–47.1	47.2–49.9		50.0–52.6	

Metroglyph analysis: For preliminary grouping of genotypes Metroglyph analysis was employed, which grouped 23 upland cotton genotypes into 7 clusters. The mean data for each character of 23 upland cotton genotypes were analyzed by this classificatory analysis (Table 3) and the class intervals are given in Table 4. A scatter plot diagram (Fig.1) was obtained by analyzing the mean data, which showed cluster I has the maximum number of genotypes comprising PB-62, PB-70, PB-54, PB-67, MNH-886, PB-64-a and PB-69. The overall cluster index score was 123 (Table 5) which is highest among all other clusters formed by Metroglyph analysis. In cluster II six genotypes PB-58, PB-59, PB-71, PB-63, PB-66 and PB-60 were included, ranked second as the cluster index score was 96. Cluster III contains three varieties viz. PB-39, PB-61 and FH-114, with total score of 69. This cluster significantly differs from other clusters formed by Metroglyph with respect to seed cotton yield, bolls per plant, fiber uniformity and fiber strength. In this cluster FH-114 showed maximum

mean values for bolls per plant and seed cotton yield among all 23 genotypes in experiment, while PB-39 had the maximum genotype index score. All the varieties included in the cluster III had the maximum array values for lint index, seed cotton yield, bolls per plant, fiber length and fiber strength. In cluster IV only two genotypes PB-65 and PB-57 were present, with individual score 22 and 21 respectively, while the cluster index score was 43. The genotypes in this cluster resemble with cluster III genotypes as their individual genotypic scores and most characters are in higher index values. In cluster V two genotypes PB-74 and PB-75 were placed having 18 as individual genotypic score value while the cluster's overall score was 36. Cluster VI had 2 genotypes CIM-496 and PB-72; the individual score of the genotypes were 13 and 17 respectively, whilst total cluster index score was 30. Cluster VII only had one genotype namely PB-64. The individual genotype score was 19 and so was the cluster index score.

Table 5: Cluster number, index score and genotypes included in each cluster following Metroglyph technique.

Cluster No.	Genotypes	Cluster Index Score
I	PB-62, PB-70, PB-54, PB-67, MNH-886, PB-64-A, PB69	123
II	PB-58, PB-59, PB-71, PB-63, PB-66, PB-60	96
III	PB-61, FH114, PB 39	69
IV	PB-65, PB-57	47
V	PB-74, PB-75	41
VI	CIM 496, PB-72	30
VII	PB-64	19

Table 6: Principal component analysis and factor loadings of different yield and quality traits in cotton genotypes.

	PC-1	PC-2	PC-3	PC-4
Eigen value	3.1494	1.8784	1.3598	1.0108
% of total variance	35.0	20.9	15.1	11.2
Cumulative Variance %	35.0	55.9	71.0	82.2
Variable	PC-1	PC-2	PC-3	PC-4
Lint index	0.328	-0.516	-0.239	-0.010
Seed index	0.134	-0.654	-0.236	0.010
Boll weight	-0.322	-0.325	-0.046	-0.536
Number of bolls	0.503	0.174	-0.177	-0.030
Fiber length	0.376	-0.027	0.303	0.401
Fiber strength	-0.261	-0.290	0.169	0.590

Fiber Uniformity	0.168	-0.055	0.615	-0.422
Fiber fineness	0.167	-0.268	0.572	-0.030
Seed cotton yield	0.502	0.107	-0.168	-0.153

Principal component analysis: In present set of experiment, out of nine, four principal components (PCs) with an Eigen value of >1 were extracted. These four principal components (PCs) shares 82.2% towards the total variability amongst cotton genotypes assessed for different yield and fiber related traits (Table 6) meanwhile remaining components contribution was only 18.8% of total variability for this set of cotton genotypes. Contribution of PCI was most towards the variability (35%) followed by PC II (20.9%), PC III (15.1%) and PC IV (11.2%). Characters including seed cotton yield, number of bolls per plant and fiber length showed considerable positive factor loadings on

PCI whilst boll weight and fiber strength has maximum negative loading (Table 6). The PC-II was characterized by only number of bolls and seed cotton yield with positive factor loading while all the other traits in this PC showed negative loadings. The PC-III elaborated due to variation among genotypes for fiber length, fiber uniformity and fiber fineness and with their positive and negative factor loadings were observed for lint index, seed index and bolls per plant. The PC-IV was explained by variability among accessions for fiber strength and fiber length with positive factor loadings, while boll weight, fiber uniformity and seed cotton yield exhibited negative loadings.

Table 7: Cluster analysis of various traits in upland cotton germplasm.

Variables	Cluster-I	Cluster-II	Cluster-III
Lint index	3.90	4.70	5.40
Seed index	7.80	7.80	8.00
Boll weight	4.10	2.80	3.10
Number of bolls	15.40	33.80	47.60
Fiber Length	26.00	27.50	29.10
Fiber strength	29.10	28.30	25.30
Fiber Uniformity	47.90	45.70	50.20
Fiber Fineness	4.70	4.70	5.00
Seed cotton yield	56.60	95.30	146.60

Table 8: Cluster membership of various cotton genotypes.

Cluster	No. of Genotypes	Genotypes
Cluster-I	10	PB-74, PB-75, PB-65, PB-62, PB-58, PB-59, PB-70, PB-66, PB-72, PB-60
Cluster-II	12	PB-54, PB-57, PB-67, MNH-886, PB64-A, PB-71, PB-63, CIM-496, PB61, PB-64, PB-69, PB-68
Cluster-III	1	FH-114

A PC biplot (Fig. 2) showed genotypes and variables are super imposed as vector on plot. The distance of each trait with respect to PCI and PCII showed the contribution of these traits in variation of genotypes used. As a whole the biplot figure showed that bolls per plant, seed cotton yield and fiber length contributed maximum towards diversity in germplasm.

Cluster Analysis: By using cluster analysis, 23 genotypes/lines were distributed into 3 clusters on the basis of various traits (Table 7). Cluster analysis exhibited that Cluster-I comprised of 10, cluster-II with 12 while cluster-III only contain 1 genotype (Table 8). Cluster-I contained genotypes with reasonable values for boll weight, fiber strength and fiber uniformity. Similarly cluster-II comprised

of genotypes having higher values for fiber strength, lint index and seed cotton yield whilst lower values were recorded for boll weight and fiber uniformity (Table 7). On the other hand, cluster-III was elucidated by maximum values for seed index number of bolls per plant fiber length, fiber uniformity and seed cotton yield. While for other traits in this cluster, selection could not be made.

DISCUSSION

Association between different traits is an important phenomenon to establish cotton improvement strategy on sound footings (Ali *et al.*, 2009). In present study, simple correlation coefficient showed some associations among the

studied traits. Lint index exhibited a direct positive association with seed index, and positive correlation was found with seed cotton yield. Boll weight, on the other hand, showed negative correlation with number of bolls per plant and negative association with fiber length. Number of bolls per plant were found to be positively correlated with some quantitative traits (Table 2) which are confirming the findings of Salahuddin *et al.* (2010), who reported positive correlation between quantitative traits of cotton, like seed cotton yield, number of bolls per plant and ginning out turn. Clement *et al.* (2011) reported negative association among yield and fiber quality traits in upland cotton. Which showed that fiber length and fiber strength had negative association with seed cotton yield, whilst positive correlation was observed with fiber maturity.

Anderson's (1957) metroglyph is a relatively simple technique used for preliminary grouping of genotypes (Bhadra and Akhtar, 1991). Which uses index score assigned to each character recorded in each line/variety, and the available germplasm is classified into different groups on the basis of total index score. According to this technique, 23 genotypes of upland cotton were formed in seven distinct groups (Table 5). Out of these 7 groups/clusters, the cluster 1 had maximum 7 genotypes with cluster index score 123. Cluster III contain three varieties viz PB-39, PB-61 and FH-114. The total score of the cluster was 69 and among these three genotypes PB-39 had maximum individual genotypic score. The individual genotypic score of this cluster summarized that all the varieties in this cluster have great potential for yield and fiber quality traits and hence could be used in cotton breeding programs (Table 3). The present findings were also validated by Rashid *et al.* (2007), as they reported deviations observed by metroglyph technique for the number of clusters formed, number of accession in each cluster and placement of the genotypes within the cluster which pointed out the accuracy and refinement of this method in accessing the germplasm. The use of metroglyph technique in the present study found sufficient backing of previous work, like on cotton (Shakeel *et al.*, 2008; Shakeel *et al.*, 2011), sugarcane (Mujahid *et al.*, 2001), rice (Cheema *et al.*, 2004) and brassica (Khan *et al.*, 2005). On the other hand, Singh and Narayanan (1993) have explained that genotypes to be used as parents in the hybridization program should be selected from distinct groups depicting total of the genetic diversity present in the germplasm.

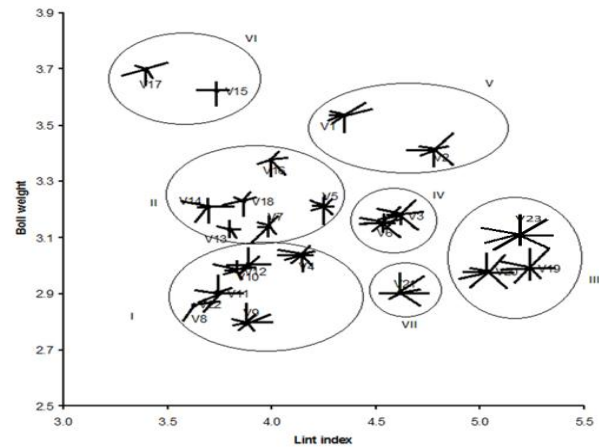


Figure 1: Scatter plot diagram of 23 genotypes of *Gossypium hirsutum* L.

Principal component (PC) is a powerful technique which provides facilitation for obtaining appropriate parental lines in order to initiate successful breeding program (Nazir *et al.*, 2013). Our results based upon principal component analysis eventually validated ample amount of variation for studied traits that could be further utilized for designing a successful cotton breeding program aimed at improving yield and fiber quality traits (Table 6). Various researchers have used this technique to validate the genetic divergence studies. The present study results were on the same line as with Saeed *et al.* (2015), who used principal component and cluster analysis on cotton crop which provides facilitation in grouping of genotypes and identification on the basis of better fiber quality. They also reported maximum contribution of first two principal components towards total variability. Ashokkumar and Ravikesavan (2011) and Malik *et al.* (2011) also reported ample amount of genetic diversity in colored cotton genotypes that allows facilitation for grouping of colored cotton genotypes. Our findings are also in agreement with that of Nazir *et al.* (2013) who concluded that contribution of first two PCs are important in total variation. Exploitation and conservation of genetic resources could be made by portioning of total variance into number of components, moreover it also provides an opportunity for utilization of most appropriate germplasm in future crop improvement program to improve specific plant traits.

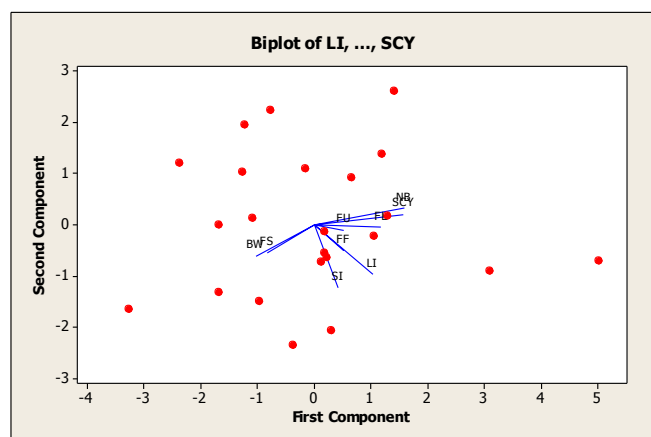


Figure 2: Biplot between PC-1 and PC-2 showing contribution of various traits in variability of cotton germplasm.

For the present set of genotypes, cluster analysis corroborates ample amount of genetic diversity among various economic value traits of upland cotton. The genotypes in cluster II and III showed better values for seed cotton yield, number of bolls per plant, lint index and fiber length (Table 7). Although cluster I had genotypes with better boll weight and fiber strength but some genotypes included in this cluster showed lower values for seed cotton yield, number of bolls per plant and fiber length. Therefore, it can be concluded that genotypes in cluster II and III can be utilized in cotton breeding programs aimed at improving yield and fiber quality traits. The scatter diagram of PC biplot revealed association among different clusters which could be due to large number of genotypes expressing variation in adaptation or some kind of similarity between them. However, cluster analysis brings altogether those genotypes expressing considerable potential for higher yield related traits at one place and likewise on the basis of fiber quality traits at another place. The findings of Amurrio *et al.* (1995) and Rabbani *et al.* (1998) were on the same line as with our results because they reported lack of association among various clusters based on agronomic characters of genotypes in peas (*Pisum sativum*) and mustard (*Brassica juncea*) respectively. The occurrence of wide diversity among clusters is of great value in order to select genotypes with better yield and fiber quality among cotton germplasm. Similar kind of findings for grouping of germplasm has been reported by Grenier *et al.* (2001).

Conclusion: In conclusion, the characters showing direct positive correlation amongst each other may be further evaluated through early generation selection. Based on biplot and correlation analysis number of bolls, seed cotton yield, boll weight, seed- and lint-index can be utilized as selection criteria for breeding program of high yielding cotton varieties. Overall, characters including seed cotton yield, number of bolls, seed index and fiber length showed higher values among different clusters and can be used as selection criteria to enhance seed cotton yield in total.

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